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ECOLOGY AND THERMAL INACTIVATION OF MICROBES
IN AND ON INTERPLANETARY SPACE VEHICLE
COMPONENTS

Twenty-sixth Quarterly Report of Progress

Research Project R-36-015-001

July 1, 1971 - September 30, 1971

Conducted by

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Introduction

In the 25th Quarterly Report, we presented data showing that 90°C was a more effective temperature than 125°C for the destruction of *Bacillus subtilis* var. *niger* when the head-space moisture was fixed at 474 μg H_2O/ml. These data have been confirmed and are summarized in Figure 1. We also investigated the influence of head-space moisture at 90°C, and observed that as the head-space moisture was increased to 474 μg H_2O/ml, there was a corresponding increase in lethality of the system. This moisture level corresponded to 100% relative humidity at 90°C, and additional water in the cans should have had no effect. We did observe, however, a profound decrease in the destruction of the organisms when additional water was added. These data are presented in Figure 2 as a point of reference for this report.

The discontinuity observed in these data is troublesome and clearly must be resolved as the first step in an orderly exploration low-temperature inactivation of *B. subtilis* var. *niger*. Initially, we postulated that by using a sealed-can system the resulting pressure changed the "saturation" moisture level, and that we were experiencing the maximum lethality at 50-75% R.H. instead of 100% R.H. Careful study of the influence of the pressure on saturation vapor pressure clearly indicated that this could not be the case and that some other explanation was necessary. The solution to the problem lay in an observation of Morrell and Scott (1) and in some preliminary data collected in this laboratory, which suggested that water vapor was more effective than liquid water for destruction of spores with all other conditions being the same.

I. EXPERIMENTAL

All experiments reported here were carried out in our conventional system. The spores were suspended in 95% ethyl alcohol, diluted in sterile double-distilled water, and dispensed with a repeating dispenser in 0.1-ml amounts in stainless steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 206 mm x 300 mm tin cans. Thirty cups were on each shelf and four shelves were used in each can for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 minutes at 45 to 50°C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 minutes for the first 70 minutes, and this was followed by five consecutive purges of nitrogen with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven and cooled to about 30°C in the equilibration hood. Appropriate amounts of water were placed in each can. The cans were sealed and removed from the equilibration hood. Heat treatments were applied as discussed below.

Spore survivors were assayed by sonifying the cups in peptone water, and plating and counting on TGE agar. Prior to heat treatment,

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(1) Morrell, W. G., and W. J. Scott. 1966. The Heat Resistance of Bacterial Spores at Various Water Activities. J. Gen. Microbiol., 43:411. (See Figure 7.)

the seams on each can were soldered and wiped to preclude leakage of water vapor during heating cycle.

II. RESULTS AND DISCUSSION

Sets of cans were prepared as described above. Each can contained 6 inoculated cups in adjacent positions on one of the middle shelves. After drying, 0.01 ml H_2O was placed in 3 of the inoculated cups, the cans sealed, and placed in an oil bath at 90°C. This amount of water was sufficient to allow the head-space moisture to come to 100% R.H., and also leave behind residual liquid water in contact with the spores to which water was added. Cans were removed at hourly intervals, and cooled. The inoculated cups were removed and then assayed for survivors. The results of this experiment are summarized in Figure 3. It is seen that the samples which were exposed to water vapor only were destroyed in less time than those containing excess liquid water. These data suggest that water vapor penetrates the spore wall with greater ease than liquid water at the same temperature.

These findings are consistent with the current concepts of intermolecular attraction between water molecules in the liquid state, which results in liquid water having an "effective" molecular weight many times greater than water vapor.

We then reviewed carefully the experimental procedures used for generating the data in Figure 2. Here, the cups were inoculated in the conventional manner, placed on one of the middle shelves, and dried. The water (in amounts indicated in the figure) was placed in the bottom of the can. We reasoned under these conditions that as the cans were heated, the cups in the center of the can would be colder than the bottom of the can. This would allow condensation to take place in the cups. When excess water was in the bottom of the can, there would be no reason for the water condensed in the cup during heating to be driven off after the can reached the desired temperature. Under these conditions, the spores would be in contact with liquid water and would be comparable to spores heated in water in Figure 3. Thus, they would be more resistant that those exposed only to enough water to make the relative humidity 100%.

To test this hypothesis, cans were prepared in the conventional manner and the inoculated cups were placed on the top or bottom shelf. The water was placed in cups near the center of the can on the middle shelves as this region would be expected to be the coldest portion of the can.

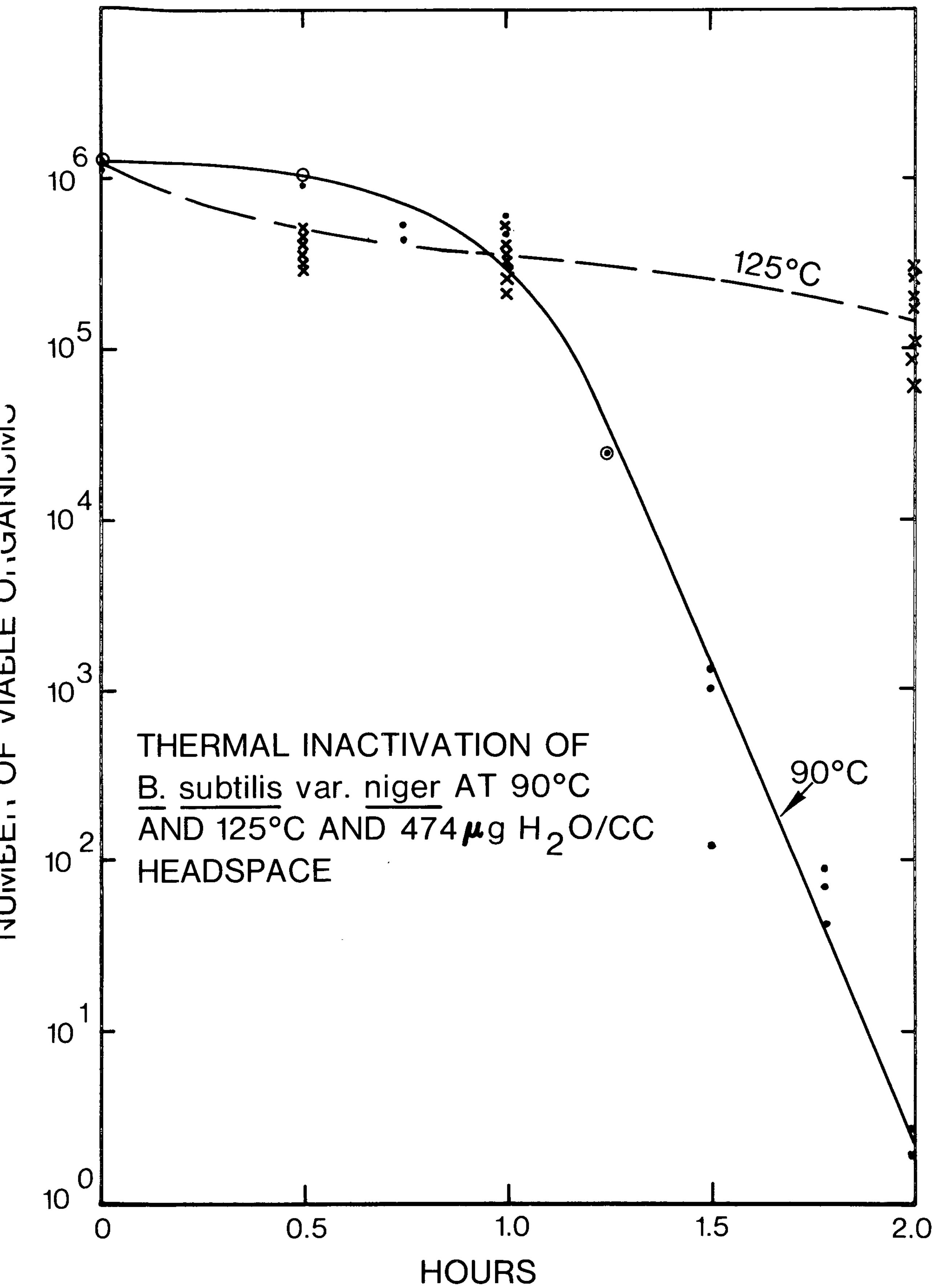
Under these conditions, there should be no condensation in the spore-containing cups, and the excess water would remain where it was placed. It would then be expected that excess water would have no effect on the system. The data presented in Figure 4 clearly demonstrate that under the conditions described above, excess water does not interfere with the effectiveness of the system and, accordingly, suggest the correctness of the hyposthesis.

These studies have provided a satisfactory explanation for the discontinuity observed in the lethality of the system when excess water was added (over that required to provide 100% R.H.), and confirms other earlier observations that the water vapor (at 100% R.H.)

is more effective than liquid water for the destruction of *B. subtilis* var. *niger*.

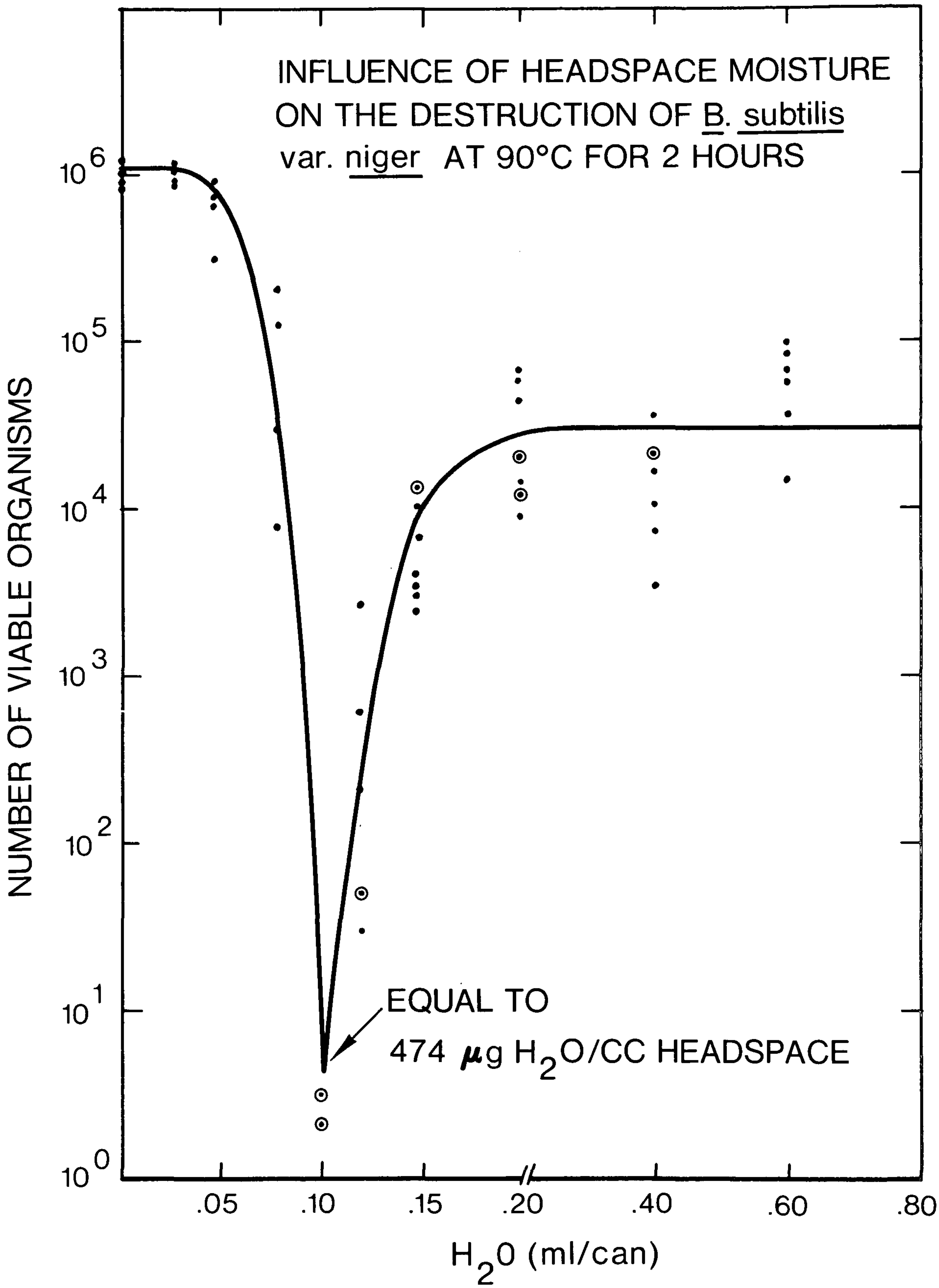
III. PROJECTED RESEARCH FOR THE NEXT QUARTER

During the next quarter, a systematic study will be made on the influence of temperature and head-space moisture on the destruction of *B. subtilis* var. *niger* at several temperatures.



NUMBER OF VIABLE ORGANISMS
10^6
10^5
10^4
10^3
10^2
10^1
10^0
125°C
90°C
THERMAL INACTIVATION OF
B. subtilis var. niger AT 90°C
AND 125°C AND 474 μg H_2O/CC
HEADSPACE
0
0.5
1.0
1.5
2.0
HOURS

FIGURE 1.



INFLUENCE OF HEADSPACE MOISTURE
ON THE DESTRUCTION OF B. subtilis
var. niger AT 90°C FOR 2 HOURS
NUMBER OF VIABLE ORGANISMS
10^6
10^5
10^4
10^3
10^2
10^1
10^0
EQUAL TO
474 μg H_2O/CC HEADSPACE
.05
.10
.15
.20
.40
.60
.80
H_2O (ml/can)

FIGURE 2.

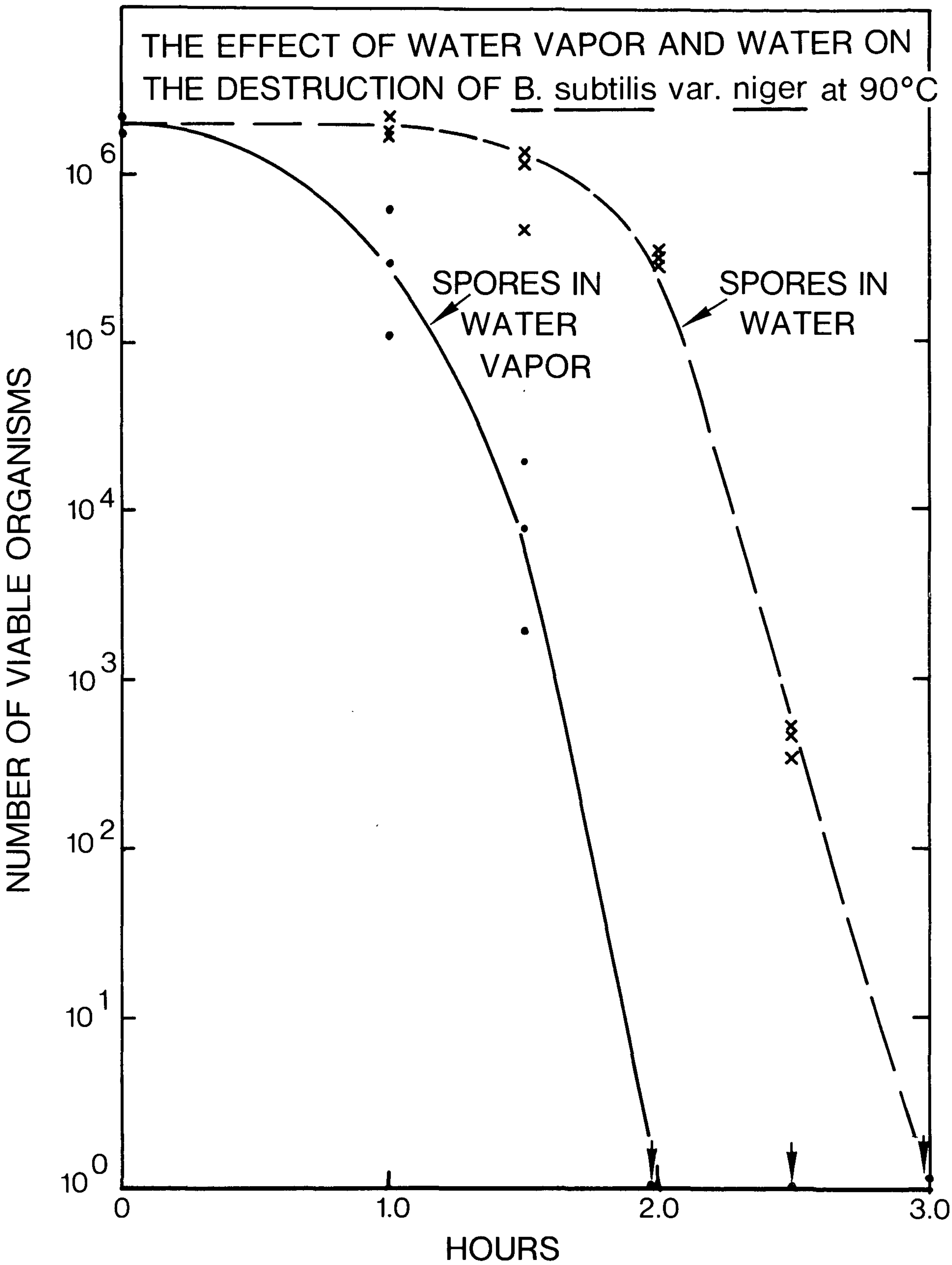

THE EFFECT OF WATER VAPOR AND WATER ON THE DESTRUCTION OF B. subtilis var. niger at 90°C
SPORES IN WATER VAPOR
SPORES IN WATER
NUMBER OF VIABLE ORGANISMS
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
1.0
2.0
3.0
HOURS

FIGURE 3.

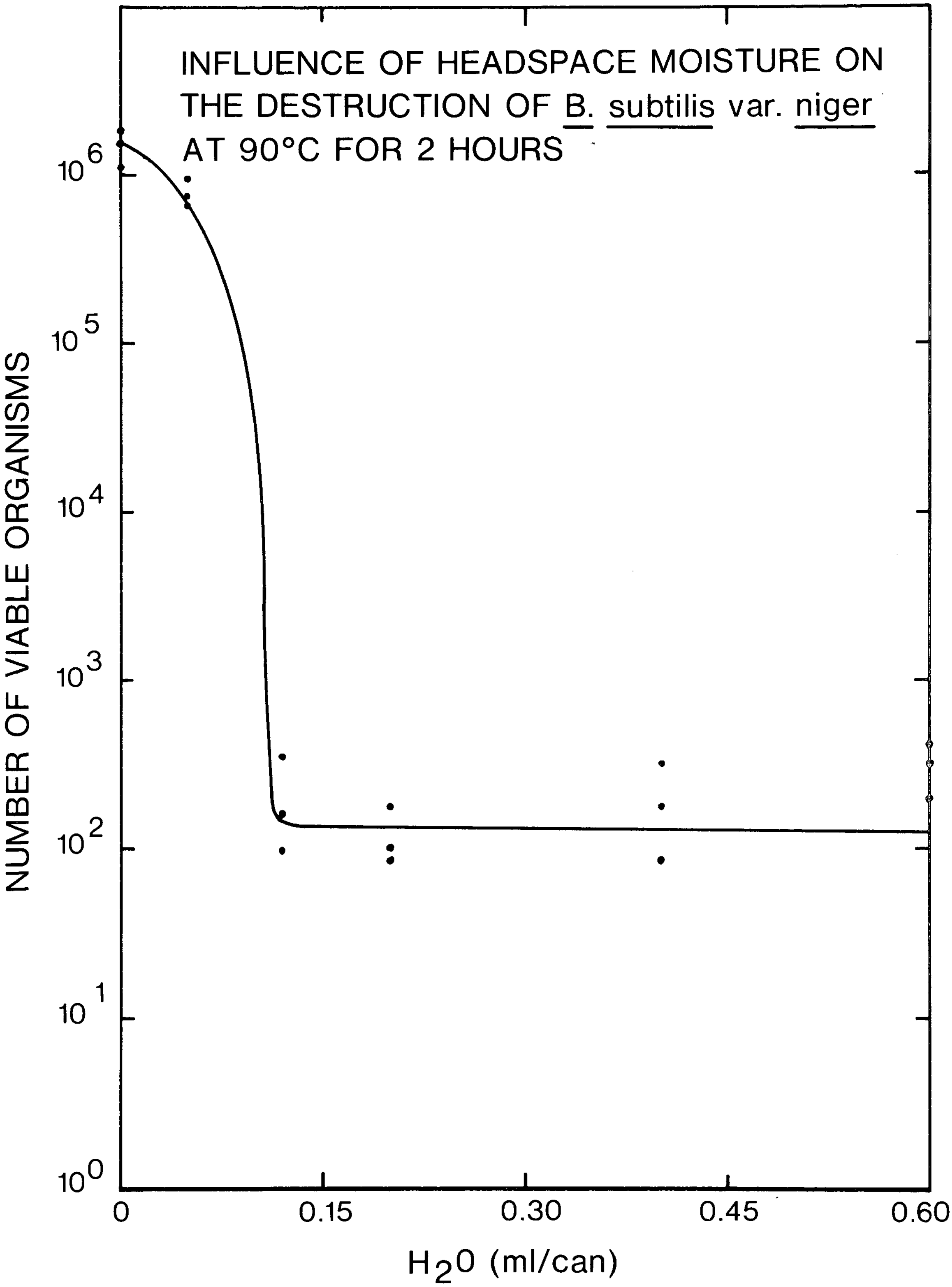

INFLUENCE OF HEADSPACE MOISTURE ON THE DESTRUCTION OF B. subtilis var. niger AT 90°C FOR 2 HOURS
NUMBER OF VIABLE ORGANISMS
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
0.15
0.30
0.45
0.60
H_2O (ml/can)

FIGURE 4.